The Occurrence of Phosphatidylethanolamine and Glycosyl Diglycerides in Thermophilic Bacilli

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Phosphatidylethanolamine, a common constituent of the polar lipids of Gram-negative bacteria, is consistently found in only one genus of Gram-positive bacteria, the bacilli (Goldfine, 1972). Gram-positive bacteria commonly contain diglycosyl diglycerides (Shaw & Baddiley, 1968; Shaw, 1970) which often occur together with phosphatidylethanolamine in the bacilli. An interrelation between phosphatidylethanolamine and glycosyl diglycerides has been demonstrated in the lipids of several bacilli (Minnikin, Abolrahimzadeh & Baddiley, 1971*a,b*, 1972). We have now shown that one strain of *Bacillus stearothermophilus* contains glycosyl diglycerides but no phosphatidylethanolamine, whereas another strain contains phosphatidylethanolamine but no glycolipids.

METHODS

Freeze-dried organisms of a strain of *B. stearothermophilus* were provided by Professor H. L. Kornberg and Dr R. K. Sundaram and had been grown (55 °C, 50 mM-sodium succinate as carbon source) essentially as described previously (Sundaram, Cazzulo & Kornberg, 1969). *Bacillus stearothermophilus* NCIB8157 (NCA1518) and the above strain were grown in a broth medium (Minnikin *et al.* 1971*a*) at 55 °C for 16 h; harvested bacteria were washed with NaCl (0.85 %) and freeze-dried.

Lipids were extracted with chloroform-methanol (2:1, v/v) and examined by two-dimensional thin-layer chromatography using a modification of the system described by Minnikin & Abdolrahimzadeh (1971); Merck silica gel H and a second development solvent, chloroform-acetic acid-methanol-water (130:27:18:7, by vol.), were used in the present study. Spraying with a saturated solution of potassium dichromate in sulphuric acid followed by charring at 200 °C revealed spots corresponding to all the polar lipids. Specific spray reagents for lipid phosphate (Dittmer & Lester, 1964), α -glycols (Baddiley, Buchanan, Handschumacher & Prescott, 1956) and free amino groups (ninhydrin in butanol) were also used.

Controlled hydrolysis of lipids with alkali was carried out as described by White & Frerman (1967). Acid hydrolysis of lipids was performed by heating in 2 M-HCl at 100 °C for 3 h in a sealed tube; the hydrolysate was evaporated to dryness over KOH in a vacuum desiccator. Water-soluble phosphates resulting from alkaline hydrolysis were examined by descending paper chromatography using the solvent systems propan-1-ol-ammonia (sp.gr. 0.88)-water (6:3:1, by vol.) (Hanes & Isherwood, 1949); butan-1-ol-pyridine-water (6:4:3, by vol.) (Jeanes, Wise & Dimler, 1951) was used for the separation of glycosides and hexoses. Material was detected on paper chromatograms by the periodate-Schiff

Fig. 1. Two-dimensional thin-layer chromatogram of the polar lipids of *B. stearrowthermophilus* provided by H. L. Kornberg and T. K. Sundaram. Plates were prepared from a slurry of Merck silica gel H (40 g) in 100 ml aqueous sodium acetate (0.2%). Developing solvents in the first direction were chloroform-methanol-water (65:25:4, by vol.), and in the second direction chloroform-acetic acid-methanol-water (130:27:18:7, by vol.). Lipids were revealed by charring plates sprayed with dichromate-sulphuric acid reagent. PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; MGDG, monoglucosyl diglyceride; DGDG, diglucosyl diglyceride.

reagents for α -glycols (Baddiley *et al.* 1956), ninhydrin reagent, and acid molybdate for phosphates (Hanes & Ishwerood, 1949). A sample of glucosylglycerol was prepared from the lipids of *Pseudomonas diminuta* as described previously (Wilkinson, 1969) and diglucosylglycerol was obtained from *Staphylococcus aureus* (Brundish & Baddiley, 1968).

RESULTS AND DISCUSSION

A thin-layer chromatogram of the polar lipids from *B. stearrowthermophilus* provided by H. L. Kornberg & T. K. Sundaram is shown in Fig. 1. Chromatograms treated with specific spray reagents showed no ninhydrin-positive lipids, but two components with the staining properties of glycolipids were observed; the phospholipid components corresponded in chromatographic mobility and staining properties with phosphatidylglycerol and diphosphatidylglycerol. Bacteria of this strain cultivated on a broth medium showed essentially the same lipid pattern. The pattern from *B. stearrowthermophilus* NCIB8157 showed components corresponding to phosphatidylglycerol, diphosphatidylglycerol, phosphatidylethanolamine (Minnikin *et al.* 1972) and an unidentified phospholipid; no glycolipids were detected. Paper chromatography [propan-1-ol-ammonia (sp.gr. 0.88)-water] of the water-soluble deacylated products from the lipids of both strains gave spots which corresponded to di(glycerylphosphoryl)-glycerol and glycerylphosphorylglycerol. The deacylation product from the lipids of *B. stearrowthermophilus* NCIB8157 contained a component which

co-chromatographed with glycerylphosphorylethanolamine; no spot corresponding to the deacylation product of the unidentified phospholipid was detected. Examination by paper chromatography (butan-1-ol-pyridine-water) of the glycosides from the first strain of *B. stearothermophilus* showed that these components stained identically to and chromatographed with the glucosylglycerol and diglucosylglycerol standards; acid hydrolysis of the lipid yielded glucose as the only sugar identified by paper chromatography in the same system.

The lipids of *B. stearothermophilus* 2184 (Card, Georgi & Militzer, 1969; Card, 1973) and *B. stearothermophilus* B65 (Oo & Lee, 1972) included phosphatidylethanolamine and phosphatidylglycerol. Long & Williams (1960) suggested that phosphatidylethanolamine (cephalin) was absent from *B. stearothermophilus* NCA1518 (NCIB8157), but we found it to be present. Glycolipids were not investigated in the previous studies, but the results of Oo & Lee (1972) suggest that they were absent from the lipids of *B. stearothermophilus* B65.

The apparent inability of a strain of *B. stearothermophilus* (Fig. 1) to synthesize phosphatidylethanolamine is remarkable as this lipid has almost invariably been encountered in bacilli (Goldfine, 1972). A phosphatidylethanolamine-deficient mutant of *Bacillus subtilis* (Marburg) has, however, been obtained (Beebe, 1971) and certain continuous cultures of *B. subtilis* (Marburg) lack this lipid (Minnikin *et al.* 1972). The absence of glycolipids from *B. stearothermophilus* NCIB8157 is surprising since most bacilli also contain glycosyl diglycerides (Shaw, 1970). Cultures of *Bacillus cereus* T have occasionally been found to contain no detectable amounts of glycolipids (Minnikin *et al.* 1971*a*). The present results support the proposed interchangeability of phosphatidylethanolamine and glycosyl diglycerides in the membranes of bacilli (Minnikin *et al.* 1971*a, b*, 1972) and suggest that polar lipid analysis may be a valuable chemotaxonomic technique for the classification of thermophilic bacilli.

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